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Mycorrhizal endogonaceae and their seasonal variations in a Greek sand dune

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With 5 figures

Synopsis: *Original scientific paper*
In a sand dune at Chalkidiki, North Greece, *Glomus macrocarpum* and *Acaulospora laevis* were present in roots of some ammophilous plants. The amount of external mycelium, spores and root infection varied with season and sand – dune zone. Highest amount of spores occurred in summer, while highest percentage root infection occurred in spring. Most abundant extramatrical mycelium was found in spring. Most plants were mycorrhizal but not all species were extensively colonized. *Pinus halepensis* was the most heavily infected species, possibly with *Glomus macrocarpum* in back of dune.
Key words: Sand dune zone, Greece, external mycelium, spores, root infection, season.

1. Introduction

Seasonal variations of vesicular – arbuscular mycorrhizas and endogonaceous fungal species are poorly known and few field studies have been undertaken e.g. GIOVANNETTI (1985), GEMMA & KOSKE (1988) and GEMMA *et al.* (1989).

This study concerns the occurrence and identity of the endophytes of sand dune plants on the coast line of Greece.

The aims of the investigation were to identify the vesicular – arbuscular mycorrhizal (VAM) fungal species active in a sand dune, to determine their distribution in two sand dune zones, to find biomass variations of rhizosphere external mycelium and spores and to assess the seasonal variations in mycorrhizal infection in 12 plant species.

2. Site Material and methods

2.1. Study site

This survey was conducted on a shore (23° 38′ E, 30° 59′ N), in Cassandra Peninsula (fig. 1), near Pefkohorion village in Chalkidiki, North Greece.

The characteristics of the sand dunes surveyed are given in table 1. Landward from the dune ridge, silt, clay, $CaCO_3$, and potassium increased. The sand, phosphorus, C/N, *p*H and sodium decreased,

Table 1. Soil physical and chemical characteristics.

Site	Particle size mass%			Extractable									Extractable cations (me/100 g of soil)		
Site	Sand	Silt	Clay	P mg/100 g of soil	*p*H	Water holding capacity mass%	$CaCO_3$ %	C %	Organic matter %	N %	C/N		Mg^{2+}	K^+	Na^+
A	94.7	0.2	5.1	0.49	8.2	1	4.2	0.08	0.14	0.03	2.7		2.04	0.22	1.50
B	92.5	1.4	6.1	0.07	7.8	1	6.2	0.09	0.16	0.04	2.3		2.01	0.37	0.75

while carbon, organic matter, nitrogen and magnesium were similar. The water holding capacity was equal in sites A and B.

The climatic data of the investigated area (fig. 2) concerning precipitation and air temperature are derived from LIVADAS and PENNAS (1975) and PENNAS (1976).

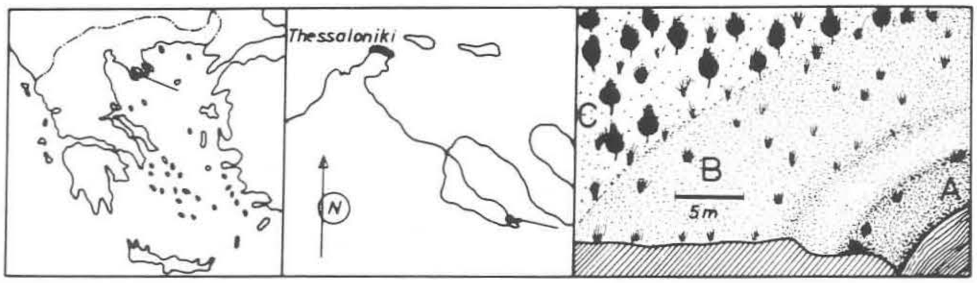

Figure 1. Map of the area investigated and succession of sand and vegetation zones in a beach near Pefkohorion.

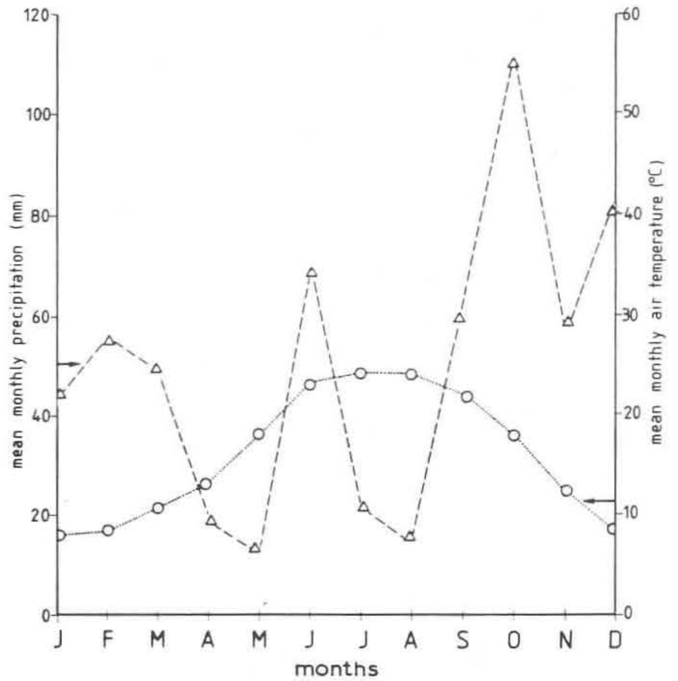

Figure 2. Climatic diagram at the hotel Xenia Paliouri. △---△, precipitation; ○····○, air temperature.

2.2. Sampling

2.2.0. General

Sand dune samples were taken from rhizosphere sand of zone A (ridge of dune) and B (back of dune). On all sampling dates two rhizosphere sand samples each ca. 2L of 12 dominant plant species from site B and two plant species of site A, were collected at random down to 20 cm depth, excluding the 2 cm top sand. The rhizosphere samples were placed in plastic bags, closed to conserve moisture and brought to the laboratory, where they were kept at 5 °C until further processing.

Root samples of 30 plant species from zones A and B were collected on July 1984.

Root samples of the 12 dominant plant species of site B were collected during the seasons of 1984 (on 20 Jan., 25 April, 15 July and 12 Oct.).

2.2.1. *Pedological analyses*

The closely adhering rhizosphere sand was collected during the seasons of 1984. Rhizosphere sand samples collected from sites A and B on 15 July 1984 were air – dried and analyzed as follows: **(a)** *p*H, measured in a 2:1 distilled water: sand suspension, using a glass electrode *p*H meter. **(b)** Soil texture determined by the pipette method. **(c)** Organic matter content was measured by WALKLEY & BLACK's (1934) method. **(d)** Nitrogen was determined by KJELDAHL's procedure. **(e)** Extractable phosphorus was determined by OLSEN's method (PAPAMIHOS & ALIFRANGIS, 1985). **(f)** The water holding capacity was determined using a pressure membrane apparatus. **(g)** Extractable cations Mg_2^+, K^+, Na^+ were determined with ammonium acetate at *p*H 7. **(h)** $CaCO_3$ was measured by the method of ALLISON & MOODIE (1965).

2.3. Identification of isolates

Spores of endomycorrhizal fungi were obtained by GERDEMANN & NICOLSON's (1963) method, picked under a dissecting microscope, transferred to glass slides, mounted in lactophenol – cotton blue, categorized, characterized, and identified according to HALL & FISH (1979) and GERDEMANN & TRAPPE (1974).

2.4. External hyphae and spores

Hyphae and spores retained on different sieves (GERDEMANN & NICOLSON, 1963) were counted and the results expressed as numbers of spores and length of hyphae per 1 liter of sand. Then, the volume of total hyphae and spores was found by considering the spores as spheres and mycelia as cylinders.

By multiplication of volumes by a specific density factor of 1.5, the fresh biomass of total hyphae and spores per 1 liter of sand was determined (PARKINSON *et al.*, 1971).

2.5. Collection and examination of roots

The root systems of four plants of each species were gently washed, cleared in 10% KOH, washed with tap water, bleached with 10% H_2O_2, washed, acidified with 1% HCl, stained with 0.01% acid fuchsin – lactophenol, drained and examined (KORMANIK *et al.*, 1980). About one hundred aproximately 1 cm segments of roots for each plant species were mounted in acid fuchsin – lactophenol on microscope slides to score the presence or absence of vesicles, arbuscules or hyphae. Then, the mean percentage to mycorrhizal infection per root segment was estimated.

3. Results

3.1. Mycorrhizal fungi and distribution

Clomus macrocarpum TUL. & TUL., one of the widely destributed *Glomus spp.* over the world from coastal lowlands to mountains (GERDEMANN & TRAPPE 1974), was the most commonly occurring in sand samples throughout the year. Chlamydospores of this species are yellow – brown, smooth, mostly subglobose, 95–210 μm diam. (fig. 3). Its sporacarps are globose to ellipsoid and up to 10 mm diam.

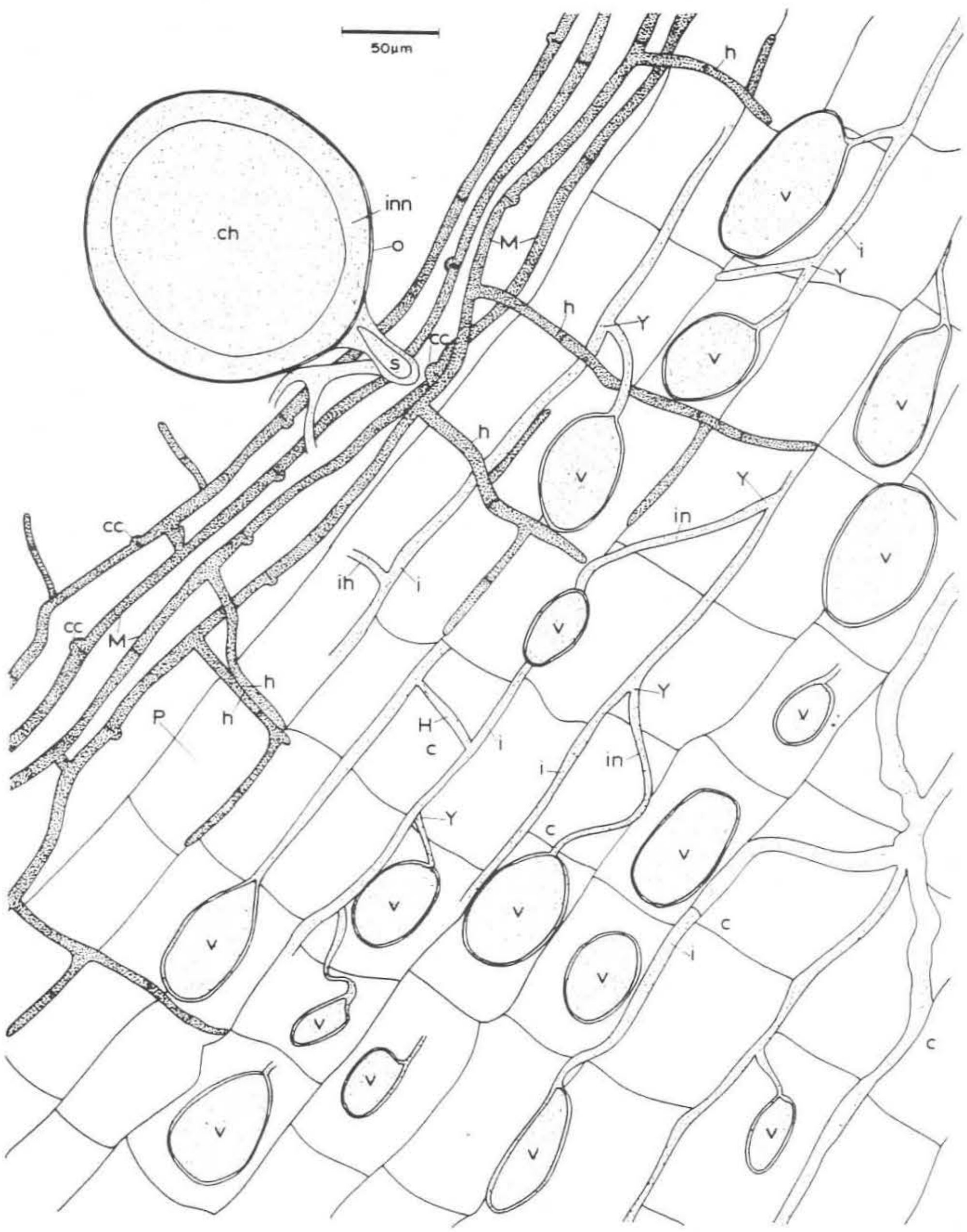

Figure 3. Root segment of *Pinus halepensis* showing combined association of VA mycorrhiza and ectomycorrhiza: c. corticial cells; P. piliferous layer; v. vesicles; i. intercellular hyphae; in. intracellular hyphae; H. H-connection; Y. Y-connections. ch. chlamydospore; o. outer wall of chlamydospore; inn. inner wall of chlamydospore; s. subtending hypha; M. Mantle hyphae; cc. clamp connection; h. intercellular hyphae of the Hartig net.

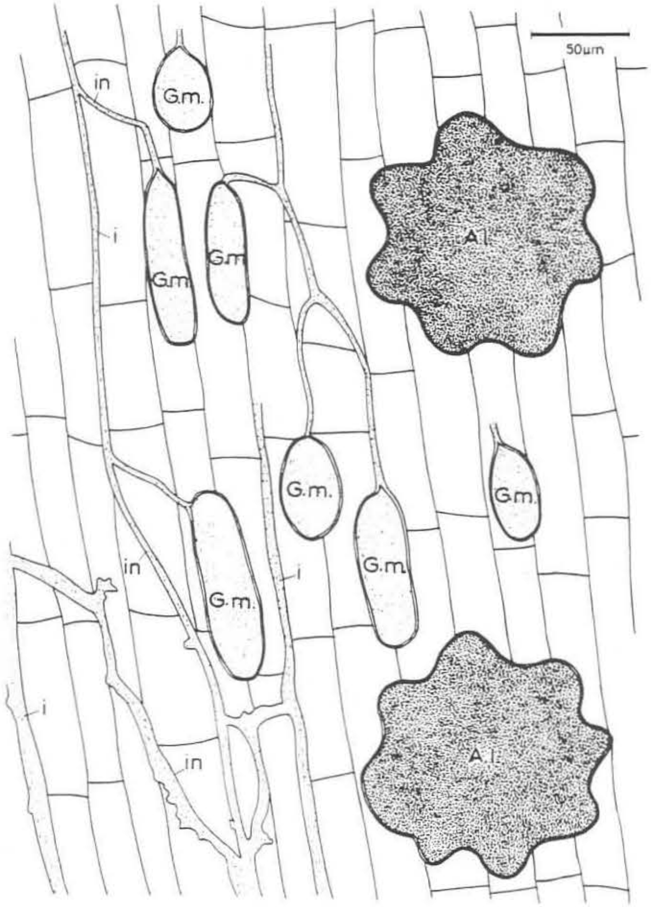

Figure 4. Longitudinal section of a mycorrhizal *Calystegia soldanella* rootlet showing: G.m., vesicles of *G. macrocarpum*; A.l., vesicles of *A. laevis*; i, intercellular hyphae; in, intracellular hyphae.

Acaulospora laevis GERDEM. & TRAPPE was the second most fragment species.

Azygospores of this species are smooth, globose, yellow and 125 – 150 µm diam. Its vesicles are lobed (fig. 4).

Both the above species were present in all seasons in zones A and B but different in number density from zone to zone and from season to season.

Other mycorrhizal species were not found in the sand samples.

3.2. Fungal hyphae and spores

The amount of external mycelium increased from winter to spring (when its peak was observed). It decreased during summer, then increased a little in autumn, remaining higher than in winter (fig. 5).

Generally spore fresh mass (fig. 5) was low in the sand dune throughout the year. External total spore production recovered reached a maximum in summer and decreased through autumn and winter to spring, when it was at a minimum (fig. 5). The number of viable spores (containing cytoplasm) was lower than that of dead spores (empty or gas filled in most sample. More nonviable spores were present in winter. The spore populations were

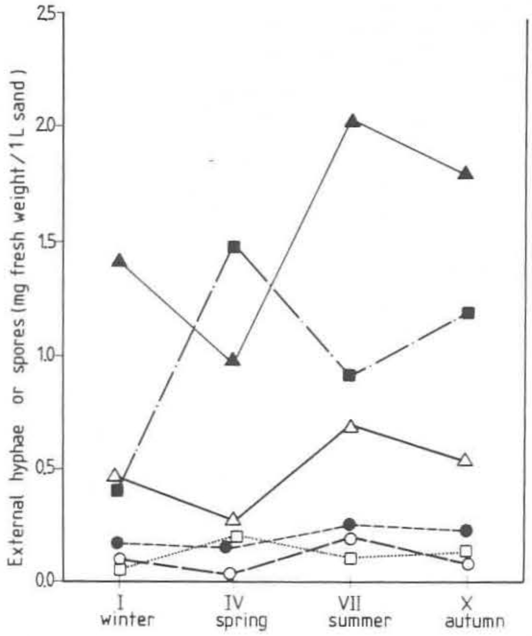

Figure 5. External mycelium and spore biomass from rhizosphere at each site of sand dune. Site A: △——△ spores of *Glomus macrocarpum* ○----○ spores of *Acaulospora laevis*, □····□ hyphae. Site B: ▲——▲ spores of *G. macrocarpum*, ●----● spores of *A. laevis*, ■----■ hyphae.

greater in site B than in A in all seasons. The front of the dune, (Zone A) which is rather mobile, had low spore numbers and the spores were of smaller size.

Spore biomass of *G. macrocarpum* was always higher than that of *A. laevis*.

3.3. Plant species and occurrence of mycorrhiza

Two zones of vegetation were distinguished. The first (zone A) was 5 m wide and closest to the shore, had scattered herbaceous plants of *Salsola kali* L. and *Euphorbia chamaesyce* L.

The second (zone B) was 15 m wide and had the following herbaceous, shrub and tree dominant species: *Agropyron sp.*, *Anthyllis hermanniae* L., *Cistus monspeliensis* L., *Crithmum maritimum* L., *Eryngium maritimum* L., *Euphorbia paralias* L., *Otanthus maritimus* (L) HOFFMANNS. & LINK, *Pancratium maritimum* L., *Pinus halepensis* MILLER, *Polygonum maritimum* L., *Sarcopoterium spinosum* (L). SPACH and *Thymus capitatus* (L.) HOFFMANNS & LINK.

Adjacent to it was a vegetation consisting mainly of *Cistus monspeliensis* L., *Pinus halepensis* and *Pistacia lentiscus* L. the density and diversity of plant species present increased with the distance from the shore.

Of the 30 plant species examined on 15 July 1984 from zones A and B, 25 species were mycorrhizal. These species had frequencies of VAM infection of 0–10, 10–20 and >20% (table 2). The following plant species were non-mycorrhizal: *Salsola kali* L., *Jasione heldreichii* BOISS *et* ORPH., *Chondrilla juncea* L., *Plantago arenaria* WALDST. *et* KIT. and *Claucium flavum* GRANTZ.

The percentage VAM infection of plant species found in site A was very low when compared with site B (table 2).

During a repeated investigation of 12 dominant plant species at site B (table 3), the following trends were observed. The maximum VAM colonization appeared in spring,

Table 2. Analysis of percentage VA mycorrhizal infection in 30 plants of zones A and B on 15 July 1984. Groups of species with different rates of mycorrhizal infection.

0–10%	10–20%	>20%
Anthyllis hermanniae L. (4.6)*	*Crithmum maritimum* L. (19.1)	*Agropyron sp.* (27.3)
Calystegia soldanella (L.) R. BR. (9.2)	*Otanthus maritimus* (L.) HOFFMANNS & LINK (14.9)	*Cichorium intybus* L. (52.3)
Cyperus capitatus VANDELLI (6.9)	*Pancratium maritimum* L. (15.2)	*Cistus monspeliensis* L. (28)
Daucus carota L. (3.0)	*Thymus capitatus* (L.) HOFFMANNS & LINK (12.7)	*Pinus halepensis* MILLER (29)
Eryngium maritimum L. (8.9)		*Sarcopoterium spinosum* (L.) SPACH (20)
Euphorbia chamaesyce L. (1.4)		
E. paralias L. (7.8)		
E. peplis L. (1.9)		
Glaucium corniculatum (L.) J. H. RUDOLPH (5.3)		
Heliotropium suaveolens BIEB. (2.3)		
Polygonum maritimum L. (8.9)		
Portulaca oleracea L. (2.1)		
Scolymus hispanicus L. (4.1)		
Silene vulgaris (MOENCH) GARCKE (2.4)		
Sonchus asper (L.) HILL (1.2)		
Verbascum pinnatifidum VAHL (5.3)		

* The numbers in parentheses mean the percentage VAM root infection.

except in two species (*Cistus monspeliensis* and *Sarcopoterium spinosum*) which showed their maxima in summer. The mean % VAM colonization increased from winter to spring and decreased in summer; it remained almost constant in autumn. Thus it appeared that colonization varied seasonally.

Generally the root infection observed was not very high in the investigated area, but "dense colonization" (>50%) occurred in two individual plants *Agropyron sp.* and *Pinus halepensis* (table 3).

Infection forms observed were arbuscules, vesicles and hyphae. Vesicles of *G. macrocarpum* appeared more frequently in almost all root infection.

Vesicles of *A. laevis* appeared only in roots of *Polygonum maritimum* L., *Portulaca oleracea* L. and *Calystegia soldanella* (L.) R. BR. In roots of the last plant species a mixture of vesicles of *G. macrocarpum* and *A. laevis* appeared (fig. 4).

Table 3. Seasonal analysis of VA mycorrhizal infection in the dominant perennial herbs, shrubs and a "tree" from site B, expressed as percent of colonized root segments.

Plant species	Winter	Spring	Summer	Autumn
Agropyron sp.	15.3	58.6	27.3	31.7
Anthyllis hermanniae	1.3	7.2	4.6	4.9
Cistus monspeliensis	5.3	19.2	28.6	24.7
Crithmum maritimum	11.9	25.2	19.1	21.8
Eryngium maritimum	0.3	12.3	8.9	6.2
Euphorbia paralias	3.5	10.7	7.8	9.8
Otanthus maritimus	6.9	21.3	14.9	7.3
Pancratium maritimum	9.8	19.5	15.2	16.7
Pinus halepensis	24.7	63.1	29.5	35.2
Polygonum maritimum	11.2	13.8	8.9	10.6
Sarcopoterium spinosum	3.1	16.9	20.4	17.0
Thymus capitatus	3.9	15.9	12.7	7.3
Mean % VA for each season	8.1	23.6	16.5	16.1

There was an interesting association of endomycorrhiza and ectomycorrhiza for *Pinus halepensis* roots. External spores and extramatrical hyphae of *G. macrocarpum* were scattered in the surrounding soil, but the penetration point was not observed at the piliferous layer. The VA mycorrhizal infection is characterized by intraradical aseptate hyphae, arbuscules and vesicles. The presence of ectomycorrhiza is assumed from dichotomously branching of roots. Microscopic examination showed the presence of a fungal mantle composed of yellow septate interwoven hyphae with clamp connections (basidiomycetes nature of the hyphae). Lateral hyphae of mantle penetrated between the cells of piliferous layer (fig. 3).

4. Discussion

The investigated coastal sand dune is very different from inland terrestrial soils. The waterholding and base exchange capacities of sand dune are low, as is the reservoir of nutrients and moisture which are dependant upon rainfall and salt spray from the sea.

The poor development of the mycorrhiza found may be due to the low nitrogen content of sand dunes, according to HEPPER, 1983, who observed very few arbuscules and intracellular hyphae in plants grown on low-nitrogen soils. The maximum production of external spores that occurred in summer allow the fungi to survive unfavorable conditions when the growth of most plants is reduced. Plant growth is finished in summer and senescence begins at that time. A result is shortage of nutrition for mycorrhizal fungi in soil. Spore production may be favored by stress conditions: such as, **(a)** high sand temperatures, **(b)** low precipitation and low moisture, high salinity and lower supply of nutrients and **(c)** low wind velocity which results in less salt spray being blown to sand dune.

The number of external spores appears to be at a minimum during spring. Possibly they then generate and produce hyphae or infecting plant roots, die, or are destroyed by saprophytic fungi or fauna.

Maximal spore biomass coincides with summer, when photosynthetic production (WALLEN, 1980), quantity of root exudates (FLUCK, 1963) and root hormone level (TORRY, 1976) are decreased, while temperature and light are increased (FURLAN & FORTIN, 1973) and dead roots begin to accumulate (GEMMA *et al.*, 1989).

The tendency observed in VAM infection agrees with the findings by HAYMAN (1974), GRAHAM (1982) and DAFT & EL-GIAMI (1978) that greater percentage colonization results from high temperatures and light intensity. After exposure of mycorrhizal spores to root exudates, germ tube length and branching was increased. Summer reduces percentage infection mainly as a result of the partial defoliation that occurs in this season. The last probably causes reduction of VAM formation, because root exudates which sustain mycorrhizal fungi during pre- and post-infection may decrease.

The higher numbers of external spores, mass of mycelium and root infections in site B compared to site A may be due to a combination of the following factors:

(a) Finer soil texture; **(b)** lower amount of phosphorus; **(c)** denser plant cover which will become plant residue and will increase spore population and infection (SAIF, 1986). In contrast, site A is almost lacking a plant cover; it had little and scattered plants of *Salsola kali* and *Euphorbia chamaesyce*. **(d)** dominant N, NE and SE wind directions, which blow the spores from site A to site B; **(e)** lower salt spray depositions occurring in the back of the dune (site B); **(f)** lower *p*H values of B, which are more favorable for highest percent germination (GREEN *et al.*, 1976); **(g)** a more stabilized soil than in site A. Plants occurring on sand dune are in particular need of VA mycorrhizal fungi for increasing their mass and receiving higher phosphate anions (KLEINSCHMIDT & GERDEMANN, 1972). It is noteworthy that the extensive external mycelium (we calculated 50 m of total length per 1 liter sand in spring sand samples) may bind large amount of sand particles in aggregates. From these observations one can also conclude that *G. macrocarpum* was the most active and important VAM fungus found in the area investigated. *Pinus* species are known to form ectomycorrhizae with Basidiomycetes f.e. *Russula*, *Lactarius*, *Boletus* and

Amanita [LEE *et al.*, 1982]. Also the Endogonaceae *E. flammicorona* forms characteristic mycorrhiza on *Pinus radiata* seedlings (CHU-CHOU & GRACE, 1984). *G. macrocarpum* is not know to form endomycorrhizas with *Pinus* species. The chlamydospores of this fungus were found during our investigation between mantle hyphae around the rootlets of *Pinus halepensis* but penetration points of extramatrical hyphae into the piliferous layer of rootlets were not observed. However, there was a characteristic VA mycorrhizal infection, but it is not clear that this infection originated from *G. macrocarpum*.

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